UNIVERSITI PUTRA MALAYSIA

ANTIMICROBIAL AND WOUND HEALING ACTIVITIES OF LOCAL EUPHORBIA HIRTA , ACALYPHA INDICA AND .PHYLLANTHUS NIRURI

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By

REEZAL BIN ISHAK

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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DEDICATION

"This research project is dedicated to those who have endure a great deal of pain and joy for just being around me"

To my parents – Thank you for believing in me

To my brothers – Thanks for keeping me tough

To Elysha Nur Ismail – Thank you for your patience

"Your sacrifices and supports gave strength to me"

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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January 2004

Chairman: **Associate Professor Muhammad Nazrul Hakim Abdullah, Ph.D.**

Faculty: **Medicine and Health Sciences**

Herbal plants are well known for their capabilities in treating diseases for centuries. The World Health Organisation (WHO) has estimated that 80% of the world's population use botanical medicines for their primary health care needs. Some of the medicinal plants under the *Euphorbiaceae* family are *Euphorbia hirta*, *Acalypha indica* and *Phyllanthus niruri*. These plants are known as traditional remedy in many rural areas throughout the world and have been used for various purposes. This study focuses on the antimicrobial activity of aqueous and ethanol extracts of these plants against selected bacteria and fungi, and its wound healing properties on surgically incised mice. The antimicrobial test using the disc diffusion method at 20, 40 and 80 mg/ml extract-impregnated disc concentrations showed significant results ($P<0.05$) of *Euphorbia hirta* aqueous leaves and barks extracts against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Salmonella enteritidis* with inhibition zones ranged from 8.33 mm to 19.67 mm. The inhibition zones for *Acalypha indica* and *Phyllanthus niruri* aqueous leaves extracts were measured between 8.33 mm to 11.0

mm and 7.33 mm to 12.33 mm respectively, but no inhibition zones were seen with the bark extracts of both plants. Only the ethanol extracts of the leaves showed antibacterial activity. The results for *Euphorbia hirta* was the most significant ($P<0.001$) with inhibition zones ranging from 7.0 mm to 13.33 mm. Ethanol leaves extracts of *Acalypha indica* and *Phyllanthus niruri* had inhibition zones between 7.0 mm to 9.67 mm and 7.0 mm to 9.67 mm respectively. The results obtained showed comparable effects to commercial antibiotics of chloramphenicol, ampicilin, penicillin G, erythromycin, tetracycline and enrofloxacine as some of the plant extracts exhibit almost equal and even bigger inhibition zones. Antifungal activity were detected only with the *Euphorbia hirta* aqueous leaves extracts against *Candida tropicalis* with mean inhibition zones of 8.67 mm at 80 mg/ml discs concentrations. The aqueous extracts of the leaves were then tested for its wound healing properties on surgically incised mice. The extracts were applied topically over the incision and inflammatory and cicatrisation activities evaluated according to a designated scoring table. *Acalypha indica* showed the most significant results ($P<0.05$) with better reduction of inflammation and increased cicatrisation activities on the wound compared to *Euphorbia hirta* and *Phyllanthus niruri* treated mice. In comparison to the acriflavine treated mice, the results demonstrated an equally good epithelial regeneration of the skins and showed to be better than the control group (no treatment).

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

AKTIVITI ANTIMIKROB DAN PENYEMBUHAN LUKA OLEH *EUPHORBIA HIRTA*, *ACALYPHA INDICA* DAN *PHYLLANTHUS NIRURI* TEMPATAN

Oleh

REEZAL BIN ISHAK

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Pengerusi: **Profesor Madya Muhammad Nazrul Hakim Abdullah, Ph.D.**

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Tumbuhan-tumbuhan herba sangat dikenali kerana kebolehannya untuk merawat pelbagai penyakit sejak dahulu lagi. Pertubuhan Kesihatan Sedunia (WHO) menganggarkan sebanyak 80% populasi dunia menggunakan ubatan-ubatan herba untuk keperluan kesihatan. Sebahagian daripada tumbuhan perubatan di bawah famili *Euphorbiaceae* adalah *Euphorbia hirta*, *Acalypha indica* dan *Phyllanthus niruri*. Pokok-pokok ini telah diketahui sebagai ubatan tradisional di kawasan-kawasan kampung di merata dunia dan digunakan untuk pelbagai tujuan. Penyelidikan ini memfokuskan kepada aktiviti antimikrob oleh ekstrak akues dan etanol pokok-pokok ini terhadap bakteria dan kulat yang dipilih, serta kebolehannya menyembuhkan luka pembedahan pada mencit. Ujian antimikrob menggunakan kaedah penyebaran cakera pada konsentrasi ekstrak 20, 40 dan 80 mg/ml menunjukkan keputusan yang signifikan ($P<0.05$) oleh ekstrak akues daripada daun dan batang *Euphorbia hirta* terhadap *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, dan *Salmonella enteritidis* dengan zon perencatan berjarak dari 8.33 mm ke 19.67 mm. Ukuran zon

perencatan oleh ekstrak daun akues dari *Acalypha indica* dan *Phyllanthus niruri*, masing-masing adalah di antara 8.33 mm ke 11.0 mm dan 7.33 mm ke 12.33 mm, tetapi tiada zon perencatan dapat dilihat dengan ekstrak batang dari kedua-dua pokok. Hanya ekstrak daun ethanol yang menunjukkan aktiviti antimikrob. Ekstrak bagi *Euphorbia hirta* menunjukkan keputusan yang paling signifikan ($P<0.001$) dengan zon perencatan berjarak dari 7.0 mm ke 13.33 mm. Ekstrak ethanol dari *Acalypha indica* dan *Phyllanthus niruri* dilihat dengan zon perencatan di antara 7.0 mm ke 9.67 mm and 7.0 mm ke 9.67 mm. Keputusan yang diperolehi ini menunjukkan kesan-kesan yang setanding dengan ubatan antibiotik komersial seperti chloramphenicol, ampicilin, penicillin G, erythromycin, tetracycline dan enrofloxacine menunjukkan kesan yang setanding, di mana sesetengah ekstrak pokok-pokok ini mempamerkan zon perencatan yang hampir sama dan juga yang lebih besar. Aktiviti antikulat pula hanya dilihat dengan ekstrak akues dari daun *Euphorbia hirta* terhadap *Candida tropicalis* dengan purata zon perencatan sebanyak 8.67 mm pada 80 mg/ml konsentrasi ekstrak. Ekstrak akues dari daun pokok-pokok ini kemudiannya diuji untuk keupayaannya menyembuhkan luka pembedahan yang telah dilakukan pada mencit. Ekstrak-ekstrak ini telah diberikan secara aplikasi luaran pada luka tersebut dan aktiviti inflamasi dan pembentukan semula kulitnya dinilai mengikut carta penilaian yang telah direka. Ekstrak *Acalypha indica* menunjukkan keputusan yang paling signifikan ($P<0.05$) dengan pengurangan inflamasi dan peningkatan pembentukan semula kulit pada luka berbanding dengan rawatan oleh *Euphorbia hirta* and *Phyllanthus niruri* pada mencit. Perbandingan keputusan dengan rawatan acriflavine mempamerkan pertumbuhan semula epitelial yang setanding dan lebih baik daripada kumpulan kawalan (tiada rawatan).

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In the name of Allah, Most gracious, Most merciful

All gratifications are referred to Allah

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I certify that an Examination Committee met on 6th January 2004 to conduct the final examination of Reezal Ishak on his Master of Science thesis entitled "Antimicrobial and Wound Healing Activities of local *Euphorbia hirta*, *Acalypha indica* and *Phyllanthus niruri*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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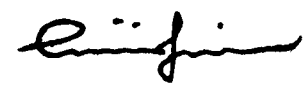

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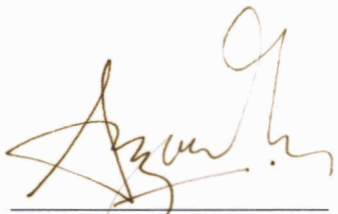
Date: 29 MAY 2004

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



REEZAL BIN ISHAK

Date: 28 MAY 2004

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LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
ATCC	American Types Cell Culture
CNS	Central nervous system
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
IBS	Institute of Bioscience
MARDI	Malaysian Agricultural and Research Development Institute
µl	Micro-litre
MIC	Minimum inhibitory concentration
mg	Milligram
ml	Millilitre
mm	Millimetre
MR/VP	Methyl Red/ Voges-Proskauer
s.d	Standard deviation
UPM	Universiti Putra Malaysia

CHAPTER 1

INTRODUCTION

The Importance of Herbal Plants

Herbal plants have been known for its capabilities in healing or treating diseases for centuries. The beginning of the nineteenth century heralded an era, in which the active secondary metabolite of medicinal plants began to be purified, with which such pure constituents were then introduced into therapy. Treatments using herbal medicines are generally safer due to its natural constituents (Perry, 1980).

In tropical countries, modern medicines are not available to most of the rural populations. The World Health Organisation (WHO) has estimated that 80% of the world's population use botanical medicines for their primary health care needs (Farnsworth *et al.*, 1985). Many common health problems in tropical rural communities including respiratory infections, diarrhoea, fungal infections, diabetes, and malaria are treated by traditional botanical medicines. Numerous tropical medicinal plant species have shown *in vivo* and *in vitro* biological activities against these health problems (Carlson *et al.*, 1997).

Euphorbiaceae

Euphorbiaceae is a family of medicinal plants often with milky juice. These plants can be seen with either alternate leaves or opposite and glands which located at the barks of the plants or at the base of the leaf-blade. It has numerous flowers which are usually small or minute. The fruit is in a capsular formation and attached together with the seeds laterally. Plants in this family are mostly found in large numbers in less expected places such as waste ground and along the drain. Some of the useful plants in this family are *Euphorbia hirta*, *Acalypha indica* and *Phyllanthus niruri*. These plants are known as traditional healer in many rural areas throughout the world. It has been used for different purposes in different places (Kirtikar *et al.*, 1975).

Euphorbia hirta

Euphorbia hirta is also popularly known as *Euphorbia pilulifera* or locally called "ara tanah" or "susun nabi". It is a small annual herb common to the tropical countries and belongs to the same family as the tic, tapioca and the rubber tree. This plant can easily be found in waste places, roadsides, gardens and rice fields. It can attain a height of 30 cm, growing erects or close to the ground (Blomquist and Oosting, 1940). *E. hirta* has a long history of usage in the treatment of various ailments. It is locally used in Africa and Australia. The plant has been used in almost every part of the world particularly Africa (Khan *et al.*, 1980).

Acalypha indica

Acalypha indica is an annual erect plant that can grow up to 30-75 cm high. It is also known as "rumput lis-lis" or "kucing galak" in Malaysia. This plant has numerous branches and flowers on their soft bark. It grows widely in fields and bushes, but preferably on a sandy and open area. *Acalypha indica* is mostly found in warm tropical areas such as Peninsular Malaysia, Thailand and Java, Indonesia (Said, 2000). This plant has been used as traditional medication for quite sometime but not widely used due to the little information known about it. Usually, the leaves of this plant are used to treat several skin infections such as scabies and ringworm (Kirtikar *et al.*, 1975).

Phyllanthus niruri

Phyllanthus niruri is a short-lived usually erect little-branched annual herb, often completely green in colour including its flowers. It has simple and very small leaves with the seeds attached along the branches below the leaves. Locally, it is known as "dukung anak" and also referred as *Phyllanthus amarus*. This plant can be found in the waste ground and is native to tropical countries (Seaforth *et al.*, 1985). *Phyllanthus niruri* is known to be used to treat various types of ailments; almost every part of the plants can be use as medication. The plant is much used as diuretic and other problems of the genito-urinary tract. The fruit is useful for wounds, scabies and ringworm remedies (Kirtikar *et al.*, 1975).

Justification of the Study

In general, this study focuses on the effects of *Euphorbia hirta*, *Acalypha indica* and *Phyllanthus niruri* as antimicrobial and wound healing agents. With alarming situation of microbial resistance against several antibacterial and antifungal drugs, alternative medicine such as medicinal plants has become a very popular source for research in recent years. Another problem face nowadays is with prolonged or incomplete wound healing process. Vast efforts are being made to discover natural agents including from medicinal plants that can expedite healing and thereby reduce other severe complications.

Objectives of the Study

1) To evaluate the antibacterial activities of the aqueous and ethanol extracts of *Euphorbia hirta*, *Acalypha indica* and *Phyllanthus niruri* against selected gram positive and gram negative bacteria.
2) To evaluate the antifungal activities of the aqueous and ethanol extracts of *Euphorbia hirta*, *Acalypha indica* and *Phyllanthus niruri* against selected moulds and yeasts.
3) To observe the wound healing activity of the aqueous extracts of the leaves of *Euphorbia hirta*, *Acalypha indica* and *Phyllanthus niruri* on surgical incision in mice.